hormone-resistant state, but the AR is a multifaceted protein and could yet hold the key to understanding all aspects of this troubling disease.

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Roles of LPA_3 and COX-2 in implantation

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Lysophosphatidic acid (LPA) is a lipid-derived G-protein-coupled receptor (GPCR) agonist that is involved in a variety of physiological and pathological processes, including cell survival, proliferation and differentiation, cytoskeletal rearrangement, cell–cell interactions, tumorigenesis and cell invasion. LPA also stimulates oocyte maturation, the preimplantation development of two- or four-cell embryos to the blastocyst stage and embryo transport in the oviduct. Recent studies revealed that targeted deletion of the LPA_3 receptor results in delayed implantation and altered embryo spacing, and significantly reduced litter size in mice. This was attributable to selective downregulation of uterine cyclooxygenase-2 (COX-2), which generates prostaglandins (PGs) E_2 and I_2. Exogenous administration of PGE_2 or the PGI_2 analogue, carba-prostacyclin, to LPA_3-deficient female mice rescued delayed implantation but did not prevent defects in embryo spacing. These findings indicate that LPA-induced COX-2 induction has a crucial role in implantation and mammalian reproduction.

Implantation

A fertilized ovum can be cultured *in vitro* independently of maternal systemic control to form an adhesion-competent blastocyst, indicating that an endogenous embryonic program coordinates its preimplantation development. However, embryonic development is much faster *in utero* than *in vitro*, possibly due to paracrine and juxtacrine signaling from the uterus. Implantation of the embryo in the uterus, an intricately timed event that requires synchronization between the embryo and a receptive endometrium, is crucial for developmental progression beyond the blastocyst stage *in vivo*. Initiation of implantation is an active biochemical process that requires a blastocyst to interact with a carefully prepared endometrium. The events of implantation include: apposition of the blastocyst to the uterine luminal epithelium; adhesion to and penetration through the epithelium and basal lamina; and invasion into the stromal vasculature [1].

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The cellular mediators of endometrial receptivity, such as cell adhesion molecules, cytokines, homeobox genes and growth factors, are regulated by female sex steroids, prostaglandins (PGs) and peptide hormones [1]. In this regard, recent studies have shown that lysophosphatidic acid (LPA), a G-protein-coupled receptor (GPCR) agonist, accelerates blastocyst differentiation through its ability to induce Ca^{2+} transients and heparin-binding epidermal growth factor (HB-EGF) autocrine signaling [2]. LPA acts on four different receptors, $LPA_{1–4}$, to produce different effects. Mice lacking LPA_1 and LPA_2 reproduce normally, but targeted deletion of LPA_3 results in delayed implantation and altered embryo spacing, embryo crowding and significantly reduced litter size [3]. These characteristics of LPA_3-deficient mice are strikingly similar to those of mice lacking cytosolic phospholipase A_2 ($cPLA_2$), an enzyme responsible for PG production through arachidonic acid metabolism.

Biological effects of LPA

LPA is a simple natural phospholipid and an important component of the cell membrane. It serves as an extracellular biomediator generated via enzymatic conversion of glycerophospholipids. The four LPA receptors have been cloned and are variably expressed in different tissues of the body. LPA_1 is abundantly expressed in testis, brain, lung, heart, spleen and intestine. LPA_2 and LPA_3 show a more restricted expression pattern and are normally found in testis, kidney, heart, lung and brain, and are overexpressed in ovarian cancer cells. LPA_3 was originally cloned from prostate cancer cells, where LPA causes proliferation by an autocrine mechanism [4]. LPA_4 levels are particularly high in the normal ovary. The widespread expression of cell-surface LPA receptors, and their coupling to several G proteins (G_q, G_s, G_i and $G_{12/13}$), leads to the regulation of several cellular processes, including cell survival, proliferation and migration, neurogenesis, vascular development, wound healing, immunity and cancer [5]. Substantial evidence is accumulating in favor of a role for the EGF receptor (EGF-R) in LPA-induced mitogenesis, cell proliferation and phosphorylation of mitogen-activated protein kinases (MAPKs). This depends on LPA-induced generation of growth-factor-like ligands, such as HB-EGF, by activation

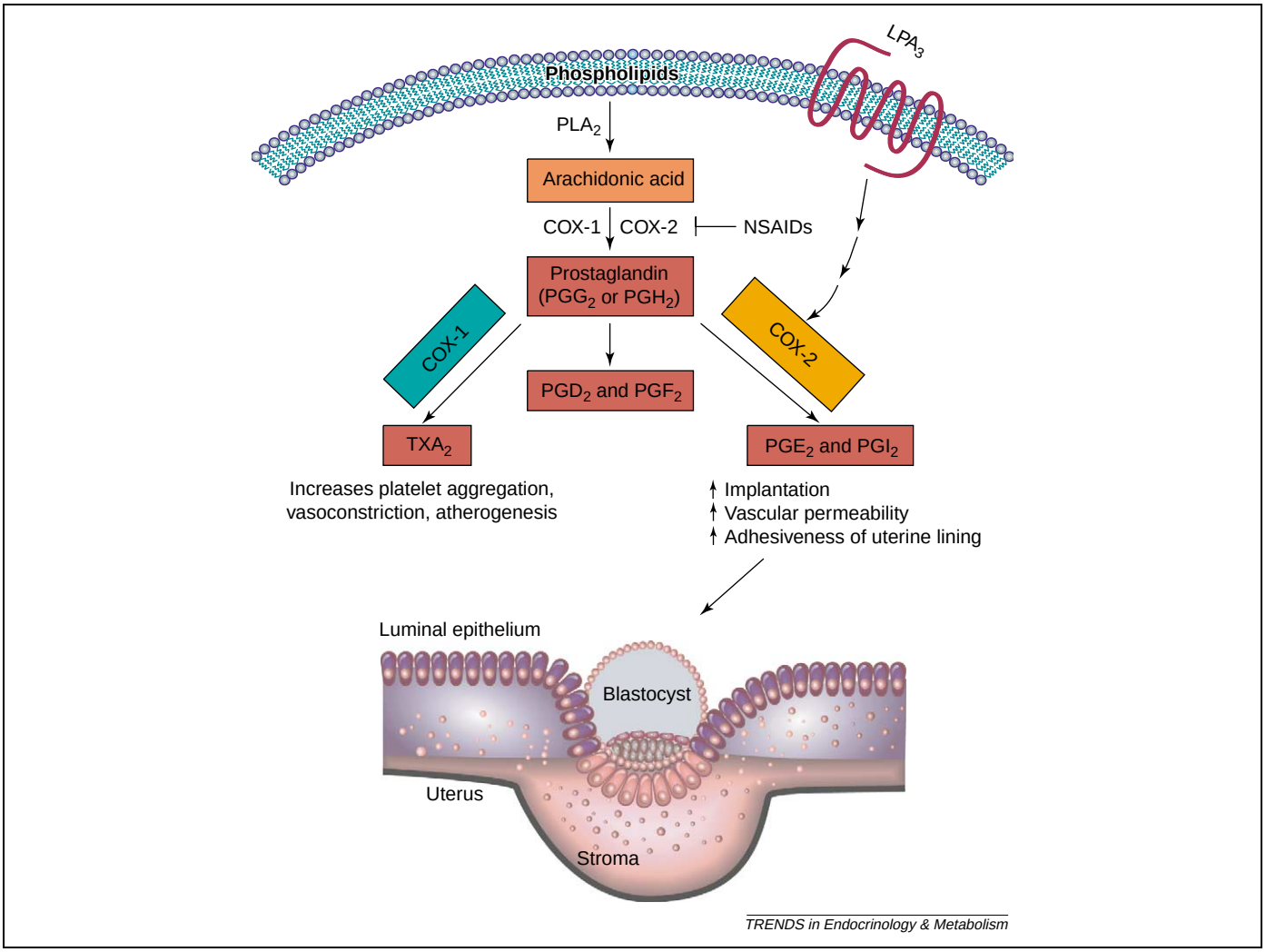

Figure 1. LPA_3-inducd COX-2 activation during implantation. $cPLA_2$ action on membrane phospholipids leads to generation of arachidonic acid, which is converted into leukotrienes and PGs by 5-lipoxygenases and COXs, respectively. Constitutive activation of COX-1 in platelets causes generation of TXA_2, which is a potent platelet activator, vasoconstrictor and atherogenic agent. COX-2 is expressed in the uterine endometrial stroma and also overlaps with LPA_3 at the site of blastocyst implantation. COX-2 induction generates PGE_2 and PGI_2, which increase vascular permeability and adhesiveness of the uterine lining. $cPLA_2$ and LPA_3 are both needed for on-time implantation.

of matrix metalloproteinases (MMPs) [4]. LPA also promotes the invasion and migration of ovarian cancer cells through MMP–EGF-R-dependent expression of cyclooxygenase-2 (COX-2) [6].

Role of COX-2 in LPA_3-mediated implantation

Recent elegant studies by Chun and colleagues [3] demonstrated that targeted deletion of LPA_3 in mice results in delayed implantation, altered embryo spacing and reduced litter size. The implantation phenotype of LPA_3-deficient mice was markedly similar to those reported for rats and mice treated with the PG-synthesis inhibitor, indomethacin [7,8], and for mice deficient in $cPLA_{2\alpha}$ [9]. Several decades of investigation have established the crucial role of PGs in the implantation process (Figure 1). Blockade of PG synthesis before or during the time of implantation causes either complete inhibition, a delay in implantation or a reduction in the number of implantation sites with diminished decidual tissue. In mice, deficiency of COX-2, but not COX-1, results in multiple female reproductive failures (including implantation defects). Conversely, enhanced COX-2, but not COX-1, expression and synthesis of COX-2-derived prostacyclin (PGI_2) are essential for implantation in the mouse [10]. A stable analogue of prostacyclin, iloprost, enhances the potential of implantation and live birth of mouse embryos [11]. These data indicate that the $cPLA_{2\alpha}$–arachidonic acid–COX–PG pathway is crucial for implantation [1].

To investigate the possible reasons for delayed implantation in LPA_3-deficient mice, Ye *et al.* [3] examined the components of PG signaling ($cPLA_{2\alpha}$, COX-1, COX-2, PGE_2 and PGI_2) and two other key regulators of implantation (leukemia inhibitory factor and Hoxa10) in the uteri of LPA_3-deficient mice. Interestingly, only COX-2 mRNA levels were significantly reduced in LPA_3-deficient mice, with decreased production of PGE_2 and PGI_2 caused by the decreased COX-2 levels. To confirm the crucial role of these PGs, the authors delivered exogenous PGE_2 and an analogue of PGI_2, cPGI, to embryonic day 3.5 LPA_3-deficient female mice. This significantly improved implantation but not the defect in embryo spacing. These results demonstrate that LPA-induced COX-2 induction has a vital role in implantation and mammalian reproduction. It is of interest that COX-2-derived PGI_2 has also been shown to confer atheroprotection [12].

Concluding remarks

There are significant mechanistic differences between rodent and human implantation. In mice and rats, rapid and eccentric implantation with apposition, attachment and invagination of the uterine epithelium occurs within six hours. After the loss of the zona pellucida, the uterine lumen closes down on the blastocyst to enhance apposition. Owing to the rapidity of these events, mice and rats are not considered to be good models for understanding the physical mechanisms of early implantation. However, the ability to exploit the vast knowledge of mouse genetics by genetically overexpressing or ablating genes in mice has been a powerful tool for elucidating gene function during implantation [13]. The study by Ye *et al.* [3] is the first to highlight a role of COX-2-derived PGs in LPA_3-induced implantation in female mice, and further investigation is needed to ascertain whether a similar mechanism operates in humans. Also, the manner in which uterine PGs coordinate with embryonic signals to mediate timely implantation remains to be determined. Recent studies have demonstrated a role for MMPs in implantation [14] and HB-EGF-induced blastocyst differentiation [2], and LPA, as well as PGE_2, are known to cause activation of MMPs and the EGF-R [15,16]. Moreover, because LPA activates COX-2 through MMP induction in ovarian carcinoma cells [6], it would be interesting to determine whether delayed implantation in LPA_3-deficient mice results from an altered expression of MMPs and HB-EGF.

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